THE RELATION OF AGE TO SIZE IN CERTAIN ROOT CELLS AND IN VEIN-ISLETS OF THE LEAVES OF *SALIX NIGRA* MARSH.

MARJORIE A. TELLEFSEN

(Received for publication June 23, 1921)

INTRODUCTION

Careful microscopic studies of the effect of age on plant structure are few. Benedict in 1915 found that structural changes in Vitis and in certain other plants are closely correlated with the age of those plants. He states that the vein-islets in the leaves of *Vitis vulpina* become smaller as the vine becomes older. This decrease in size is due to the encroachment of vascular tissue. Ensign in 1919 found that the size of the vein-islets of Citrus leaves is closely correlated with the maturity of those leaves. From the most immature to the fully matured leaves of seedlings there is a gradual increase in the size of vein-islets.

Since roots of cuttings develop from meristematic cells, the writer decided to see if the age of the trees from which cuttings are made affects the size of the cells in the roots developed from them. It was also desired that further investigations be carried on with leaf tissue, to determine in particular if the age of a tree is in any way correlated with the size of vein-islets in the leaves of that tree. A woody perennial, *Salix nigra* Marsh., was chosen for the investigation.

The black willow was selected, first, because it is abundant in the region of Cincinnati, Ohio, where the investigations were carried on, and second, because of the comparative ease with which specialized tissues, *i.e.*, roots, etc., will develop from the meristematic tissue of cuttings under laboratory conditions, thus rendering them available for study. Furthermore, the black willow was chosen because of its great ability to reproduce vegetatively as well as sexually.

Because the relation of reproductive methods to rejuvenescence has not been fully determined, the problem has lost none of its interest. Sexual reproduction is generally believed to effect complete rejuvenescence of protoplasm. The effects of asexual propagation are still discussed.

Among the lower forms of life many plants and animals reproduce asexually for a considerable length of time, and for these organisms this type of propagation seems to effect rejuvenescence. Some of the unicellular forms are known to reproduce in no other way. As organisms become more and more complex, sexual reproduction appears and in many

forms entirely replaces asexual propagation, while in other species both sexual and asexual reproduction occur.

Because sexual reproduction causes rejuvenescence, the efficiency of vegetative propagation in these more complex organisms has become a subject for investigation. The question is especially pertinent because many of the higher plants, especially cultivated forms, are propagated extensively by means of cuttings. If vegetative reproduction does not effectively rejuvenate protoplasm in these complex forms, plants grown from cuttings are as old as the plant from which the cutting was taken. The direct bearing of this question on the commercial propagation of certain cultivated plants is evident. *Salix nigra* Marsh., the species selected for the present investigation, is one in which the power of vegetative propagation is well developed, and one in which sexual reproduction occurs regularly. In such a species any relation exhibited between age and cell structure is particularly significant.

Collection and Care of Cuttings

During the fall of 1917 and the spring of 1918, cuttings were made from trees of *Salix nigra*. They were tagged, numbered, and taken to the laboratory where they were placed in warm water over night. The following day the cuttings were put in jars of tap water. Fresh water was supplied every few days. About a month later the cuttings were transferred to a galvanized iron tank of running water. A slatted arrangement made of wood floated on the water and served to hold the cuttings up and to keep them from too close contact with each other. A correct balance could not be determined between the required intake of water for aëration and the requisite temperature for root formation, so that water molds developed and killed the cuttings. However, a few roots were formed, and these were killed and imbedded for study.

During the fall of 1918 and the early spring of 1919, cuttings were made from branches of recent growth of trees of *Salix nigra*. The age of each tree was determined roughly by measuring the diameter of the main trunk of the tree six inches above the ground. The latter distance was arbitrarily chosen so that measurements would be taken a uniform distance above the ground. The trees were then classified according to their diameter. Three groups were differentiated and arbitrarily distinguished by the letters A, B, and C. Trees less than 2½ inches in diameter were considered to be in group A, those 4 to 6 inches in diameter in group B, and those 8 inches or more in diameter in group C.

Cuttings were tagged and taken to the laboratory, where they were placed in a warm bath (38°–40° C.) for two hours to stimulate protoplasmic activity. When removed from the bath, some were placed in the tank of running water. The majority of the cuttings, however, were put in glass jars which were then placed either in Wardian cases in diffuse sunlight or

on window sills supplied with bottom heat from radiators. The water in the jars was renewed as needed and entirely changed about once a month.

Benedict (1915) in writing of senile changes in physiological activities of plants says that "the most obvious characteristic is a decrease in rate of growth." McFarland mentions in a recent test that the regenerative power appears to be greater in proportion to the youth of an animal. The present writer found that cuttings from younger trees rooted in less time than those from older trees. Leaves appeared on cuttings of younger trees before they did on those of older trees.

When roots appeared on the cuttings, they were allowed to grow until about $2\frac{1}{2}$ to 3 inches long. Then sections were cut from roots of the several cuttings 2 inches back from the root tip and put into Flemming's strong solution. The sections were taken 2 inches back from the root tip in order to find differentiation of the ground meristem.

After the usual killing, washing, and dehydration, the pieces of roots were imbedded in paraffin, and both longitudinal and cross sections were cut six microns in thickness. The sections were stained in safranin and Mayer's haemalum, or in safranin and light green. After being stained, the sections were mounted in balsam.

Measurement of Root Cells

When the prepared root sections were examined, it was found that large air spaces had developed in the cortical tissue. The number of these air spaces was found not to be constant, but varying from three to five, four being the common number. This provision for aëration gave the cross sections a decided three-, four-, or five-rayed appearance, the number of xylem strands coinciding in each case with the number of large air spaces.

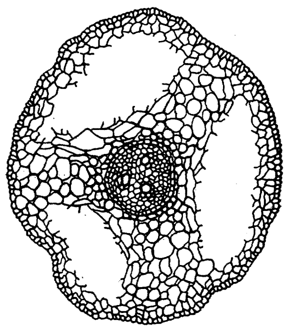

FIG. 1. Cross section of root of *Salix nigra* Marsh., showing three large air spaces and a three-rayed condition of the cortex. × 90.

Somewhat similar conditions have been reported for other plants. In 1888 Scott and Wager recorded the fact that the primary cortex of floating roots of *Sesbania aculeata* Pers. consists of rounded cells among which are very large lacunae filled with air.

Lily Batten (1918) mentions the fact that cortical root cells of *Epilobium hirsutum* are very loosely packed in young roots and that large air spaces occur in the roots of plants grown under very moist conditions.

Ada Hayden (1919) found cortical air spaces in roots of prairie plants studied.

In 1913, Norris planted *Zea Mays* in various media and examined the structure of the roots. He found that the medium used influenced root structure, particularly that of the cortex. Air spaces

appeared in those roots whose surrounding medium afforded a scanty air supply. Norris reported that the cortex of roots growing in water is of a very flimsy and indefinite nature and is apt to fall to pieces when an attempt is made to section the roots. For roots grown in sawdust he re-

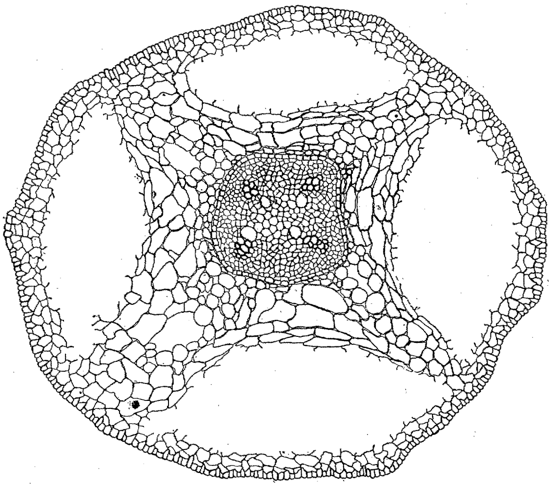

FIG. 2. Cross section of root of *Salix nigra*, showing four large air spaces and a four-rayed condition of the cortex. × 90.

ports that air spaces occur throughout and appear to be formed by the breaking down of groups of cells, the cell walls being in many cases not completely broken and stretching across the air spaces.

The conditions found in *Salix nigra* correspond to those described by Norris for *Zea Mays*. The cortical root tissue of Salix appears very loose, and, as has been stated, the air spaces are well developed. Remnants of cell walls stretching out into these air spaces lead the writer to conclude that this provision for aëration results from the breaking down of cortical cells.

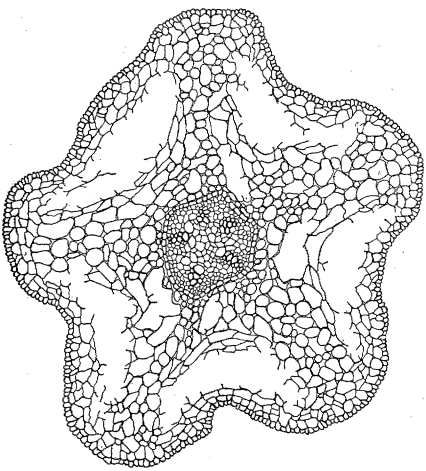

FIG. 3. Cross section of root of *Salix nigra*, showing five large air spaces and a five-rayed condition of the cortex. × 90.

When roots were removed from the cuttings to be placed in killing fluid, these air spaces appeared as continuous air passages parallel with the long axes of the roots.

The accompanying figures will serve to illustrate the kind of material used in determining the size of cells.

Five cells were selected from each tissue visible in the cross sections of about one hundred roots. The cells were chosen at random save those of the cortex. These latter were selected from the spherical cells within the cortical rays. Measurements were made in the same manner in each case. The cells were measured first through their tangential diameter and then through their radial diameter. The thickness of the cell walls was measured. All measurements were recorded in microns.

Because of the large amount of aëration tissue present, there was obvious difficulty in securing complete longitudinal sections of the roots. However, five cells were selected from the epidermis and five from the cortex of each of about thirty roots. In each case the first measurement was taken through the center of the long axis of the cell; the second measurement was made through the center of the short axis of the cell. Data were recorded in the same manner as for the cross sections.

TABLE I. *Measurements in Cross Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra Marsh., Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	12.94 x 17.26
302	$\frac{3}{16}$	11.28 x 13.61
306	$\frac{3}{16}$	13.61 x 14.94
308	$\frac{1}{8}$	12.60 x 11.28
318	$\frac{3}{16}$	11.95 x 10.29
321	$\frac{3}{16}$	12.61 x 12.28
340	−1	9.62 x 10.62
343	−1	10.29 x 12.28
357	−1	11.95 x 12.26
364	−1	10.62 x 9.29
401	2	10.29 x 10.28
606	2	8.96 x 12.28
608	2	13.28 x 11.95
616	2	9.29 x 9.29
636	$1\frac{1}{2}$	13.28 x 13.94
637	2	14.44 x 18.93
638	2	9.96 x 13.77
707	2	10.79 x 20.25
710	1	8.95 x 13.01
713	1	11.62 x 13.08
714	$1\frac{1}{2}$	10.78 x 10.95
715	2	10.78 x 16.27
716	1	8.96 x 13.44
717	2	12.28 x 13.01
719	$1\frac{1}{2}$	11.62 x 13.94
720	$1\frac{1}{2}$–2	10.29 x 13.77
724	2	11.78 x 14.60
733	1	12.39 x 12.28
734	1	11.62 x 13.61
735	2	11.95 x 15.27
737	1	9.96 x 12.82
746	1	11.22 x 15.27
Average		11.31 x 13.31

TABLE 2. *Measurements in Cross Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	14.24 x 9.25
62	4	8.95 x 10.95
68	4	10.95 x 15.93
167	4½	11.78 x 10.29
330	5	11.78 x 12.28
405	4	12.69 x 14.60
601	6	10.95 x 11.62
609	4	11.95 x 11.62
612	5	9.79 x 13.11
617	4	8.30 x 12.94
621	5	11.28 x 13.92
623	6	9.62 x 12.28
627	6	10.45 x 10.95
633	4	11.28 x 14.27
635	5	12.28 x 16.93
639	4	8.30 x 10.62
641	6	11.62 x 17.92
642	5	12.61 x 13.77
700	5	11.62 x 15.27
706	6	13.94 x 16.93
Average		11.21 x 13.27

TABLE 3. *Measurements in Cross Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	13.41 x 11.95
44	10	9.62 x 13.94
46	18	12.57 x 16.50
48	10	11.96 x 12.28
151	12	11.62 x 14.92
326	13	10.06 x 15.75
327	13	9.62 x 10.55
328	13	11.41 x 14.94
400	10+	10.29 x 14.81
404	15	7.97 x 10.61
600	8–10	9.62 x 13.55
602	20	10.46 x 13.61
604	10	13.12 x 15.07
605	8	9.13 x 10.62
611	8	10.65 x 10.79
620	8	12.28 x 15.27
624	10+	9.63 x 9.63
625	10+	9.92 x 11.79
628	8	10.13 x 11.62
629	8	9.79 x 11.29
630	10	11.95 x 10.27
631	8–10	11.45 x 12.96
632	10+	12.55 x 13.11
634	10	9.62 x 13.28
643	10	10.79 x 14.94
702	10+	10.29 x 14.61
703	10+	14.44 x 11.79
704	10+	11.79 x 13.45
705	10+	13.44 x 15.10
708	8	11.62 x 15.27
Average		10.04 x 13.14

TABLE 4. *Measurements in Longitudinal Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	74.70 x 11.28
302	$\frac{3}{16}$	114.87 x 8.63
308	$\frac{1}{8}$	58.10 x 9.96
707	2	82.21 x 12.95
710	1	124.25 x 13.28
713	1	68.06 x 10.95
714	$1\frac{1}{2}$	79.45 x 8.68
715	2	101.92 x 11.28
716	1	75.03 x 11.95
717	2	108.23 x 10.12
719	$1\frac{1}{2}$	91.96 x 9.96
733	1	93.62 x 13.28
746	1	86.20 x 8.30
Average		89.12 x 10.81

TABLE 5. *Measurements in Longitudinal Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	63.36 x 10.72
62	4	55.44 x 8.30
68	4	91.63 x 13.94
Average		70.14 x 10.98

TABLE 6. *Measurements in Longitudinal Section of Epidermal Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
46	18	75.36 x 9.29
48	10	37.85 x 8.63
91	8–10	44.08 x 14.60
600	8–10	74.70 x 9.96
702	10+	101.26 x 10.63
703	10+	83.83 x 12.45
704	10+	109.56 x 9.63
729	10+	93.50 x 15.94
Average		77.51 x 11.39

TABLE 7. *Measurements in Cross Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	1/4	20.91 x 24.56
302	3/16	27.64 x 25.31
306	3/16	25.89 x 26.89
308	1/8	23.57 x 25.89
318	3/16	27.55 x 29.71
321	3/16	22.24 x 23.90
340	–1	28.88 x 27.88
343	–1	26.22 x 22.57
357	–1	27.88 x 26.91
364	–1	29.21 x 22.90
606	2	23.24 x 22.57
608	2	25.23 x 23.57
616	2	24.56 x 26.89
636	1½	33.21 x 29.21
637	2	36.02 x 37.82
638	2	32.20 x 31.54
640	1	40.16 x 36.18
707	2	24.90 x 23.86
710	1	28.55 x 29.21
713	1	38.53 x 37.05
714	1½	36.54 x 41.16
715	2	32.87 x 33.99
716	1	27.72 x 33.86
717	2	30.54 x 27.68
719	1½	28.22 x 33.53
720	1½–2	24.90 x 28.81
724	2	32.87 x 34.86
733	1	35.85 x 38.84
734	1	33.86 x 35.52
735	2	31.87 x 34.19
737	1	31.87 x 33.86
746	1	31.54 x 40.17
Average		29.57 x 30.02

TABLE 8. *Measurements in Cross Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	16.42 x 18.58
62	4	26.56 x 28.22
68	4	21.58 x 25.56
167	4½	29.71 x 22.72
330	5	24.23 x 29.21
405	4	26.89 x 24.56
601	6	34.19 x 31.20
609	4	29.54 x 29.54
612	5	33.86 x 30.71
617	4	26.56 x 24.07
621	5	33.53 x 29.55
623	6	28.67 x 29.38
627	6	30.54 x 32.53
633	4	36.85 x 38.78
635	5	33.53 x 36.52
639	4	29.05 x 27.37
641	6	28.88 x 33.53
642	5	31.91 x 30.87
700	5	32.03 x 33.03
706	6	30.87 x 30.87
Average		29.92 x 29.36

TABLE 9. *Measurements in Cross Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	25.54 x 22.88
44	10	22.24 x 30.87
46	18	27.22 x 28.22
48	10	31.54 x 34.52
326	13	21.91 x 26.56
327	13	27.55 x 26.56
328	13	28.85 x 27.77
400	10+	24.57 x 27.55
404	15	25.89 x 29.21
602	20	28.82 x 27.68
604	10	31.87 x 33.86
605	8	22.91 x 25.23
611	8	29.88 x 37.18
620	8	28.38 x 31.04
624	10+	30.21 x 30.54
625	10+	28.12 x 30.88
628	8	21.25 x 22.91
629	8	26.56 x 26.89
630	10	24.90 x 27.56
631	8–10	34.24 x 26.84
632	10+	30.54 x 26.89
634	10	20.24 x 20.58
643	10	32.87 x 30.54
702	10+	33.54 x 34.86
703	10+	28.72 x 29.72
704	10+	29.22 x 27.56
705	10+	34.20 x 34.87
708	8	32.20 x 36.85
Average		27.28 x 29.16

TABLE 10. *Measurements in Longitudinal Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	61.08 x 13.94
302	$\frac{3}{16}$	43.49 x 12.28
308	$\frac{1}{8}$	64.74 x 29.54
707	2	150.72 x 23.57
710	1	148.50 x 22.37
714	$1\frac{1}{2}$	80.88 x 29.55
715	2	112.21 x 17.92
716	1	86.98 x 17.43
717	2	120.35 x 23.24
719	$1\frac{1}{2}$	101.59 x 32.20
733	1	66.98 x 20.58
746	1	112.21 x 27.69
Average		95.81 x 22.52

TABLE 11. *Measurements in Longitudinal Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	70.13 x 14.59
62	4	82.33 x 15.27
68	4	95.94 x 30.21
Average		82.80 x 20.02

TABLE 12. *Measurements in Longitudinal Section of Cortical Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
46	18	48.14 x 9.62
48	10	56.44 x 14.27
91	8–10	81.65 x 18.60
154	15	66.40 x 20.91
600	8–10	70.71 x 20.58
702	10+	117.86 x 23.24
703	10+	108.73 x 21.58
704	10+	112.88 x 17.60
Average		82.85 x 18.30

TABLE 13. *Measurements in Cross Section of Endodermal Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	15.93 x 10.29
306	$\frac{3}{16}$	10.95 x 11.48
308	$\frac{1}{8}$	8.30 x 9.29
318	$\frac{3}{16}$	10.27 x 8.96
321	$\frac{3}{16}$	11.62 x 9.62
343	−1	10.95 x 7.96
357	−1	15.47 x 8.30
364	−1	14.27 x 11.62
608	2	9.96 x 8.46
636	$1\frac{1}{2}$	9.96 x 10.95
637	2	12.95 x 11.62
640	1	10.62 x 8.13
710	1	9.09 x 8.63
713	1	12.77 x 8.96
714	$1\frac{1}{2}$	12.11 x 8.96
715	2	10.95 x 8.13
716	1	10.79 x 9.62
719	$1\frac{1}{2}$	11.62 x 9.62
724	2	10.95 x 8.63
733	1	11.62 x 10.29
735	2	10.12 x 10.62
737	1	9.96 x 6.37
746	1	9.98 x 8.13
Average		11.31 x 9.33

TABLE 14. *Measurements in Cross Section of Endodermal Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	9.96 x 7.12
62	4	11.29 x 9.29
68	4	9.96 x 12.28
167	$4\frac{1}{2}$	9.79 x 8.96
330	5	8.63 x 9.29
405	4	11.12 x 10.62
601	6	11.95 x 9.62
609	4	8.96 x 7.96
612	5	9.29 x 8.30
617	4	8.50 x 7.96
621	5	9.29 x 9.29
623	6	11.95 x 8.30
627	6	11.95 x 6.97
633	4	9.59 x 10.19
635	5	8.16 x 10.95
639	4	11.12 x 8.46
641	6	10.95 x 8.63
700	5	10.95 x 11.28
706	6	12.61 x 6.38
Average		10.31 x 9.04

TABLE 15. *Measurements in Cross Section of Endodermal Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	11.45 x 9.62
44	10	11.95 x 9.96
326	13	14.11 x 10.97
327	13	7.74 x 8.96
328	13	10.62 x 7.77
400	10+	9.96 x 14.77
404	15	8.61 x 7.61
602	20	9.96 x 9.15
611	8	8.79 x 9.29
624	10+	11.79 x 8.30
625	10+	8.80 x 9.79
628	8	9.63 x 12.28
630	10	9.96 x 9.13
631	8–10	8.80 x 11.29
632	10+	11.79 x 8.80
634	10	10.96 x 9.30
643	10	8.96 x 9.29
704	10+	11.95 x 9.30
Average		10.32 x 9.64

TABLE 16. *Measurements in Cross Section of Phloem Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	9.96 x 7.63
306	$\frac{3}{16}$	6.97 x 5.97
308	$\frac{1}{8}$	6.80 x 6.97
321	$\frac{3}{16}$	9.62 x 6.97
340	−1	6.64 x 7.70
343	−1	5.91 x 6.64
364	−1	7.63 x 5.97
606	2	7.80 x 7.63
608	2	7.96 x 7.63
636	$1\frac{1}{2}$	8.04 x 7.63
640	1	7.64 x 7.97
733	1	7.96 x 6.97
Average		7.82 x 7.14

TABLE 17. *Measurements in Cross Section of Phloem Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	6.76 x 6.40
62	4	5.64 x 6.64
68	4	6.30 x 7.63
405	4	7.70 x 7.63
609	4	7.96 x 7.63
633	4	6.97 x 7.13
635	5	8.07 x 7.96
641	6	6.68 x 7.63
642	5	8.13 x 7.30
700	5	6.30 x 6.30
Average		7.05 x 7.22

TABLE 18. *Measurements in Cross Section of Phloem Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	8.46 x 7.07
44	10	7.30 x 8.30
48	10	8.76 x 7.63
326	13	6.97 x 9.03
605	8	6.30 x 8.30
624	10+	6.64 x 7.30
628	8	7.49 x 6.97
630	10	5.81 x 7.64
631	8–10	8.01 x 7.47
632	10+	5.98 x 6.79
634	10	7.97 x 6.64
643	10	6.64 x 5.31
Average		7.19 x 7.37

TABLE 19. *Measurements in Cross Section of Xylem Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
76	$\frac{1}{4}$	11.62 x 14.60
302	$\frac{3}{16}$	9.13 x 8.57
306	$\frac{3}{16}$	8.30 x 6.03
308	$\frac{1}{8}$	8.30 x 7.62
318	$\frac{3}{16}$	8.30 x 10.95
321	$\frac{3}{16}$	10.62 x 8.30
340	−1	16.26 x 18.26
343	−1	8.29 x 9.96
357	−1	11.62 x 11.62
364	−1	15.27 x 15.60
401	2	8.30 x 9.95
606	2	9.29 x 11.95
608	2	14.27 x 15.27
636	$1\frac{1}{2}$	9.96 x 10.95
637	2	14.27 x 13.94
707	2	14.27 x 13.28
710	1	11.28 x 8.07
713	1	13.28 x 10.95
714	$1\frac{1}{2}$	11.62 x 13.28
715	2	9.95 x 9.44
716	1	9.15 x 11.62
717	2	8.63 x 9.62
719	$1\frac{1}{2}$	10.62 x 8.69
720	$1\frac{1}{2}$–2	7.30 x 6.64
724	2	12.08 x 12.41
733	1	13.28 x 15.93
734	1	13.61 x 13.94
735	2	9.70 x 9.62
737	1	11.25 x 11.12
Average		11.03 x 11.31

TABLE 20. *Measurements in Cross Section of Xylem Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	9.96 x 8.54
62	4	8.96 x 11.95
68	4	9.29 x 10.62
167	4½	10.95 x 11.18
330	5	9.29 x 9.29
405	4	11.62 x 12.94
601	6	7.30 x 13.28
609	4	9.62 x 10.60
612	5	11.28 x 10.95
621	5	14.94 x 14.27
623	6	8.96 x 9.46
627	6	11.28 x 6.97
633	4	12.78 x 13.61
635	5	9.40 x 12.17
639	4	15.93 x 18.92
641	6	10.79 x 10.79
642	5	10.29 x 9.62
700	5	11.95 x 11.95
706	6	10.61 x 13.28
Average		10.80 x 11.61

TABLE 21. *Measurements in Cross Section of Xylem Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	13.78 x 13.61
44	10	12.28 x 17.59
46	18	8.63 x 11.95
48	10	11.62 x 11.97
326	13	9.96 x 8.46
327	13	10.61 x 17.26
328	13	8.13 x 9.13
404	15	11.29 x 10.95
602	20	9.15 x 12.95
604	10	11.78 x 11.78
605	9	9.62 x 10.60
611	8	10.45 x 12.61
620	8	9.97 x 9.96
624	10+	11.29 x 12.95
625	10+	11.29 x 9.30
628	8	10.96 x 10.29
629	8	10.62 x 10.96
630	10	11.62 x 13.28
631	8–10	10.96 x 12.61
634	10	13.94 x 14.94
643	10	11.29 x 12.12
702	10+	13.61 x 10.63
703	10+	11.62 x 10.51
704	10+	12.28 x 9.95
705	10+	11.62 x 13.92
708	8	9.63 x 9.30
Average		11.07 x 11.90

TABLE 22. *Measurements in Cross Section of Meristematic Root Cells of Cuttings of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
364	−1	10.29 x 10.29
733	1	9.29 x 11.28
Average		9.79 x 10.78

TABLE 23. *Measurements in Cross Section of Meristematic Root Cells of Cuttings of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
58	4	11.74 x 11.39
62	4	10.95 x 13.28
68	4	7.30 x 9.62
617	4	8.96 x 10.45
641	6	12.28 x 12.61
Average		10.24 x 11.47

TABLE 24. *Measurements in Cross Section of Meristematic Root Cells of Cuttings of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Measure, 5 Cells (Microns)
35	10+	10.95 x 11.62
44	10	8.63 x 9.62
46	18	12.11 x 11.28
404	15	9.63 x 9.63
602	20	7.97 x 10.95
605	8	8.63 x 11.61
620	8	9.96 x 9.29
628	8	11.95 x 11.82
631	8–10	8.30 x 15.44
643	10	10.79 x 14.94
Average		9.89 x 11.62

The measurements of the five cells of each tissue were averaged for each root measured. The averages thus obtained were averaged again according to tissues, and a grand average of cell measurements for each tissue was obtained. For example, the measurements of the five epidermal cells were averaged for each root. These averages were then added and averaged for the number of roots examined, so that the resulting grand average represented the average measurement for all the epidermal cells. A grand average was obtained for epidermal root cells from cuttings of Groups A, B, and C. These averages were then compared and tabulated as shown in tables 25 and 26. This procedure was followed for each root tissue present.

From an examination of table 25 it will be seen that the average measurements of cross sections of root cells of cuttings from trees of *Salix nigra*

TABLE 25. *Comparison of Average Measurements (in Microns) of Cross Sections of Root Cells of Cuttings of Trees of Salix nigra*

Tissue	Group A		Group B		Group C	
	Ave. Meas. of Cells	Computed Cross Sections	Ave. Meas. of Cells	Computed Cross Sections	Ave. Meas. of Cells	Computed Cross Sections
Epidermis	11.31 x 13.31	150.5361	11.21 x 13.27	148.7569	11.04 x 13.14	145.0656
Cortex	29.57 x 30.02	887.6914	29.92 x 29.36	878.4512	27.28 x 29.16	795.4848
Endodermis	11.31 x 9.33	105.5223	10.31 x 9.04	93.2024	10.32 x 9.64	99.4848
Phloem	7.82 x 7.14	55.8348	7.05 x 7.22	50.9010	7.19 x 7.37	52.9903
Xylem	11.03 x 11.31	124.7493	10.80 x 11.61	125.3880	11.07 x 11.90	131.7330
Meristem	9.79 x 10.78	105.5362	10.24 x 11.47	117.4528	9.89 x 11.62	114.9218

TABLE 26. *Comparison of Average Measurements (in Microns) of Longitudinal Sections of Root Cells of Cuttings of Trees of Salix nigra*

Tissue	Group A		Group B		Group C	
	Ave. Meas. of Cells	Computed Longitudinal Sections	Ave. Meas. of Cells	Computed Longitudinal Sections	Ave. Meas. of Cells	Computed Longitudinal Sections
Epidermis	89.12 x 10.81	963.3872	70.14 x 10.98	769.1372	77.51 x 11.39	882.8389
Cortex	95.81 x 22.52	2,157.6412	82.80 x 20.02	1,657.6560	82.85 x 18.30	1,516.1550

differ with the age of the tree from which the cuttings were taken. As the tree grows older, the epidermal and cortical cells of the roots become smaller, while the xylem and meristematic cells of these roots become larger. The endodermal and phloem cells of the roots seem to become smaller through their tangential diameters and larger through their radial diameters.

Table 26 shows that epidermal and cortical root cells seem to become shorter with the increasing age of the parent tree.

The noted increase in size of the xylem cells in cross section is particularly interesting in the light of unpublished studies by H. M. Benedict, who found that in the leaves of *Vitis vulpina* L. the amount of vascular tissue not only increases, but also that the xylem cells become larger as the vine grows older.

Concerning *Salix nigra*, one may conclude from the material examined that the age of the tree seems to affect the size of root cells of cuttings.

Collection and Care of Leaves

During the fall of 1917 and the fall of 1918, leaves were collected from about seventy black willow trees. The age was determined as stated above

TABLE 27. *Average Areas of Vein-islets of Leaves of Trees of Salix nigra, Group A*

Number of Tree	Diameter of Tree (Inches)	Ave. Areas of 20 Vein-islets (Sq. Mm.)
8	$1\frac{1}{2}$	.244
11	$\frac{1}{2}$	.440
16	2	.305
17	1	.440
23	1	.423
24	1	.458
25	$1\frac{1}{4}$	.423
27	2	.268
29	2	.323
30	$\frac{1}{2}$	.354
31	1	.314
32	2	.423
37	2	.611
38	$2\frac{1}{2}$	.478
39	$1\frac{1}{2}$	.323
49	2	.578
51	$1\frac{1}{2}$	.458
52	$2\frac{1}{2}$	.440
70	$\frac{1}{4}$	.550
71	1	.550
72	$\frac{1}{4}$	.458
73	$\frac{1}{4}$	.610
74	$\frac{3}{16}$	.550
75	$\frac{3}{16}$	.393
77	$\frac{1}{4}$	.440
78	$\frac{1}{4}$	.687
79	$\frac{1}{4}$	.354
80	$\frac{1}{8}$	.407
81	$\frac{3}{16}$	.407
82	$\frac{1}{8}$	.440
83	$\frac{3}{16}$	.354
84	$\frac{3}{16}$	.478
85	$\frac{1}{8}$	.407
86	$\frac{1}{8}$	.478
Average		.4369

in discussing the collection of cuttings. As before, a tree less than $2\frac{1}{2}$ inches in diameter was considered to be in group A, one 4 to 6 inches in diameter in group B, and trees 8 or more inches in diameter were considered to be in group C.

TABLE 28. *Average Areas of Vein-islets of Leaves of Trees of Salix nigra, Group B*

Number of Tree	Diameter of Tree (Inches)	Ave. Areas of 20 Vein-islets (Sq. Mm.)
2	4	.261
3	5	.314
6	6	.297
7	6	.261
9	5	.275
10	5	.305
18	4	.333
21	6	.239
28	5	.261
33	5	.524
36	4–5	.524
47	5	.343
57	4	.282
59	4	.289
61	4	.379
64	4	.393
65	4	.360
66	5	.379
67	5	.220
69	5	.323
Average		.3281

TABLE 29. *Average Areas of Vein-islets of Leaves of Trees of Salix nigra, Group C*

Number of Tree	Diameter of Tree (Inches)	Ave. Areas of 20 Vein-islets (Sq. Mm.)
1	8	.382
5	8	.261
12	10	.244
13	15	.275
14	12	.244
15	10	.282
19	13	.289
20	12	.323
22	8	.330
35	10	.500
Average		.3130

TABLE 30. *Comparison of Average Areas of Vein-islets of the Leaves of Salix nigra*

Average Area of Vein-islets of Leaves of Trees, Group A	.4369 sq. mm.
Average Area of Vein-islets of Leaves of Trees, Group B	.3281
Average Area of Vein-islets of Leaves of Trees, Group C	.3130

About twenty leaves of average size were picked from each tree. They were carefully placed in envelopes each bearing the number of the tree from which they were collected.

Measurement of Vein-islets

After the leaves were taken to the laboratory, they were cut transversely at their broadest point and the cut ends of the veins and veinlets were counted. The width of the leaves was measured at the place where the veinlet count was made. All data were tabulated.

The number of cut ends of veins in the twenty leaves from each tree was added and averaged to find the average number of veinlets for the entire tree. This number was then divided by the average width of the twenty leaves to obtain the average area of the vein-islets for the whole tree. This method was used for each of the seventy trees. A grand average was then made of vein-islet areas of trees in groups A, B, and C. These areas were compared and tabulated as shown in tables 27–30.

It will be seen from an examination of table 30 that the average area of vein-islets in the leaves from trees in group C is smaller than in leaves from trees in groups A and B. The average area of vein-islets of trees in group C is 71 percent smaller than that of vein-islets of trees in group A.

Summary and Conclusions

1. Cuttings from younger trees of *Salix nigra* Marsh. rooted in less time than those from older trees.

2. Leaves appeared on cuttings of younger trees before they did on those of older trees. Age seems to be correlated with a decrease in rate of growth.

3. The number of xylem strands coincides with the number of large cortical air spaces.

4. Epidermal and cortical root cells of cuttings seem to become smaller with the increasing age of the parent tree.

5. Xylem and meristematic root cells of cuttings seem to become larger as the parent tree becomes older.

6. Endodermal and phloem root cells tend to decrease in size through their tangential diameters and to increase in size through their radial diameters.

7. The age of the parent tree apparently affects the size of root cells of cuttings from that tree.

8. The average area of vein-islets in leaves from older trees is smaller than average vein-islet areas of leaves from younger trees. With the onset of senility the amount of vascular tissue seems to increase, thus reducing the average area of vein-islets.

9. Large air spaces were found in the cortical tissue of the willow roots. The number of these spaces is not constant. Four is the average number.

The writer wishes to acknowledge gratefully the help so willingly given by many friends and colleagues in the preparation of this paper. My thanks are especially due to Prof. Harris M. Benedict of the University of

Cincinnati for suggesting the problem and for his kindly and patient direction of the work.

BIBLIOGRAPHY

Batten, Lily. 1918. Observations on the ecology of *Epilobium hirsutum.* Jour. Ecol. **6**: 161–177.

Benedict, H. M. 1915. Senile changes in leaves of *Vitis vulpina* L. and certain other plants. Cornell Univ. Agr. Exp. Sta. Memoir 7.

Brotherton, W., and Bartlett, H. H. 1918. Cell measurement as an aid in the analysis of quantitative variation. Amer. Jour. Bot. **5**: 192–206.

Curtis, O. F. 1918. Stimulation of root growth in cuttings by treatment with chemical compounds. Cornell Univ. Agr. Exp. Sta. Memoir 14.

Ensign, M. R. 1919. Venation and senescence of polyembryonic Citrus plants. Amer. Jour. Bot. **6**: 311–329.

Hayden, Ada. 1919. The ecologic subterranean anatomy of some plants of a prairie province in central Iowa. Amer. Jour. Bot. **6**: 87–105.

Lamb, G. N. 1915. Willows: Their growth, use and importance. U. S. Dept. Agr. Contrib. For. Serv. Bull. 316.

Norris, F. D. M. 1913. Production of air passages in the root of *Zea Mays* by variation of the culture media. Ann. Rep. and Proc. Bristol Naturalists' Soc. IV, **3**: 134–136.

Scott, D. H., and Wager, H. 1888. On the floating-roots of *Sesbania aculeata* Pers. Annals of Bot. **1**: 307–314.